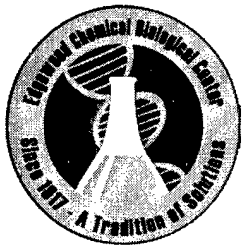

EDGEWOOD

CHEMICAL BIOLOGICAL CENTER

U.S. ARMY RESEARCH, DEVELOPMENT AND ENGINEERING COMMAND

ECBC-TN-022

SAMPLING EFFICIENCY MEASUREMENT METHODS FOR AEROSOL SAMPLERS

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14. ABSTRACT
Aerosol samplers and concentrators are characterized at the U.S. Army Edgewood Chemical Biological Center. The characterization methods are summarized and include (1) monodisperse fluorescent/nonfluorescent Polystyrene Latex (PSL) microspheres with fluorometric analysis or Coulter Multisizer® Analysis, (2) polydisperse solid aluminum oxide particles with Aerodynamic Particle Sizer (APS) analysis or Coulter Multisizer® Analysis, (3) liquid fluorescent oleic acid particles with fluorometric analysis, and (4) bioparticles with Coulter Multisizer®, culturing, enzyme linked immunosorbent assays (ELISA), polymerase chain reaction (PCR), and APS analyses. Samples are sent out for ELISA and PCR analysis. Aerosols are generated using several methods. The PSL microsphere aerosols are generated using a Collison nebulizer, sonic nozzle, Ink Jet Aerosol Generator (IJAG), and puffers. Aluminum oxide particle aerosols are generated using a sonic nozzle. Fluorescent oleic acid particle aerosols are generated using a Vibrating Orifice Aerosol Generator (VOAG). Bioparticle aerosols are generated using puffers (metered dose inhaler devices), nebulizers, IJAG, and sonic nozzle.

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PREFACE

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SAMPLING EFFICIENCY MEASUREMENT METHODS FOR AEROSOL SAMPLERS

1. INTRODUCTION

Air samplers are important in the war against terrorism and on the battlefield to detect the presence of chemical, biological, and nuclear aerosols. Samplers and detection systems must be tested and their performance efficiencies determined so that suitable samplers and detectors can be mated appropriately for various challenges. Knowledge and use of efficient air samplers enhance the ability to protect soldiers, first responders, and the general public from air borne agents.

The performance of an aerosol sampler is the product of the sampler's aspiration, transmission, and collection efficiencies. The aspiration efficiency of a sampler gives the efficiency with which particles enter into the sampler inlet. Transmission efficiency gives the efficiency with which particles are transported to the collection point, and the collection efficiency gives the efficiency with which particles are captured and retained by the sampling medium.

Samplers are characterized in wind tunnels to determine the effect of wind speed on the aspiration efficiencies of the sampler inlets. Tests are conducted in flow-through cells to determine sampler component efficiencies. Sampling efficiency tests at calm air conditions are conducted in a 70-m^3 chamber using several methods. This report only describes methods used in a 70-m^3 chamber at the U.S. Army Edgewood Chemical Biological Center (ECBC). The methods used are (1) monodisperse fluorescent and non-fluorescent polystyrene latex (PSL) microspheres with fluorometric analysis or Coulter Multisizer® analysis, (2) polydisperse solid aluminum oxide (Al_2O_3) particles with Aerodynamic Particle Sizer (APS) or Coulter Multisizer® analysis, (3) fluorescent oleic acid particles with fluorometric analysis, (4) bioparticles with Coulter Multisizer®, culturing, enzyme linked immunosorbent assays (ELISA), polymerase chain reaction (PCR), and APS analyses. The APS analysis is only used in cases where concentrated aerosol is the samplers' output. The aerosols are generated using many methods. The table below lists the generation methods, resultant size distribution, and analysis methods that are used at ECBC.

Table. Aerosols Used in Sampler Characterization Tests, Size Distribution, Aerosol Generation Methods, and Analyses Methods.

Aerosol Type	Generation Method	Size Distribution	Analysis Method
PSL	Nebulizers, Sonic nozzle, IJAG Puffers	Monodispersed 0.5 to 6 μm	Microscopy Fluorometry Coulter Counter
Fluorescent Oleic acid droplets	VOAG Spinning top*	Monodispersed 3 to 20 μm	Fluorometry
Test dust Al_2O_3	Sonic Nozzle	Poly dispersed 0.5 to ≤20 μm	Gravimetric Coulter Counter
Biosimulants	Nebulizers Puffers IJAG, Sonic Nozzle, Bubblers*	Poly, mono, or narrowly dispersed 1 to ≈20 μm	Culturing Coulter Counter, APS

*Spinning top aerosol generators and bubblers are not routinely used at ECBC for sampler characterization.

Sampling efficiency tests are conducted by generating the aerosol for a specified time, mixing the aerosol in the chamber for 1 min to achieve a uniform aerosol concentration in the chamber, and then sampling from the chamber using the samplers and reference filters. The collected samples are analyzed, and the sampling efficiency is determined by comparing the particles collected by the sampler to particles collected by the reference filters. The airflow rate of reference filters and samplers, and the liquid volumes of samples and the reference filters are considered in the sampling efficiency calculations. For example, in a test using fluorescent particles, the sampling efficiency is calculated using the following equation:

$$\text{Sampling Efficiency} = \frac{\left[\frac{(\text{fluorometer reading of sampler}) \times (\text{liquid volume})}{(\text{air flow rate})}\right]}{\left[\frac{(\text{fluorometer reading of reference filter}) \times (\text{liquid volume})}{(\text{air flow rate})}\right]} \times 100 .$$

To achieve accurate sampling efficiency results, the airflow rates must also be measured carefully. The airflow rates of the reference filters and samplers are measured using a Buck calibrator (A.P. Buck, Incorporated, Orlando, FL), a Kurz airflow meter (Kurz Instruments, Incorporated, Monterey, CA), a TSI mass flow meter (TSI, St. Paul, MN), or a DryCal (BIOS International Corporation, Butler, NJ). The ECBC Calibration Laboratory calibrates all flow meters annually. Other characteristics of the samplers are also measured including weight and dimensions. Power usages are measured using a power meter (Extech Instruments, Taiwan).

2. METHODS

Chambers and equipment used in characterizing samplers at ECBC are described in this section. A 70-m^3 chamber is used in most of the sampler characterization tests. There are also smaller stainless steel and Plexiglas chambers available if very high concentration aerosols are needed for low airflow rate sampler tests.

2.1 70-m^3 Chamber.

Sampler characterization tests are conducted in a 70-m^3 Biosafety Level 1 (BL 1) chamber, Figure 1. Temperature and humidity of the chamber can be set and maintained easily and accurately by a computer. This computer also controls the power receptacles inside the chamber.

High efficiency particulate air (HEPA) filters are installed at the inlet to filter air entering the chamber to achieve very low background particle concentrations in the chamber. Similarly, HEPA filters are installed at the exhaust port to filter all particles leaving the chamber. The chamber aerosol is cleaned by exhausting the chamber air through the HEPA filters, and by pumping HEPA-filtered air into the chamber. Approximately 2×10^4 L/min is the maximum amount of airflow that can be exhausted from the chamber by the exhaust pump. There is also a small recirculation system that removes air from the chamber, passes it through a HEPA filter, and delivers it back to the chamber. This system is useful when the aerosol concentration in the chamber needs to be reduced incrementally.

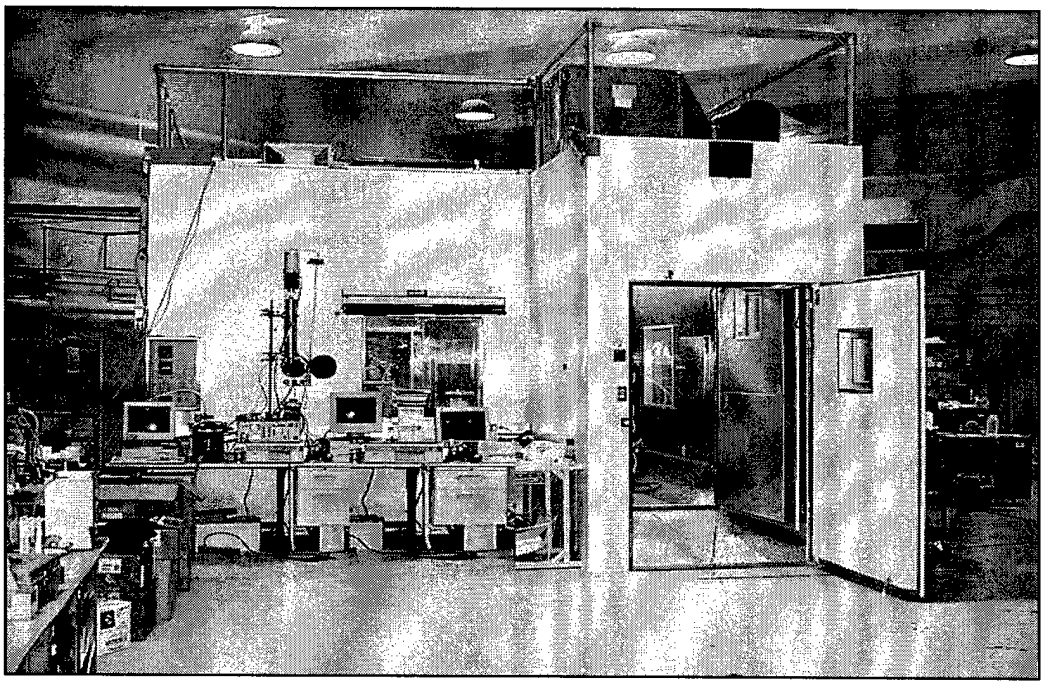

Figure 1. 70-m^3 Biosafety Level 1 Chamber Used at ECBC.

Aerosols can be either generated inside the chamber or generated outside and delivered to the chamber. To achieve a uniform aerosol concentration in the chamber, fans mix chamber air after and/or during the aerosol generation. Previous tests showed that mixing the aerosol in the chamber for 1 min is adequate to achieve a uniform aerosol concentration.

2.2 Nebulizers.

Both 36- and 24-jet Collison nebulizers (BGI Incorporated, Waltham, MA), Figure 2, are used at ECBC to generate the PSL aerosols. The PSL microspheres are added to deionized water and are used in the nebulizer. The PSL concentration in liquid is low so that doublet and triplet PSL aerosols are not generated. The Collison nebulizer is connected to compressed air, and the compressed air exits at high velocity from small holes inside the nebulizer. The low pressure created in the exit region by the Bernoulli effect causes liquid to be drawn from the bottom of the nebulizer through a second tube. The liquid exits the tube as a thin filament that stretches out as it accelerates in the air stream until it breaks into droplets. The spray stream is directed onto the wall where larger droplets are impacted and removed from the air. The PSL microspheres are carried out of the nebulizer. Particles generated by this method are charged, and are neutralized by passing them through a Model 3054 Kr-85 radioactive neutralizer (TSI Incorporated, St. Paul, MN).

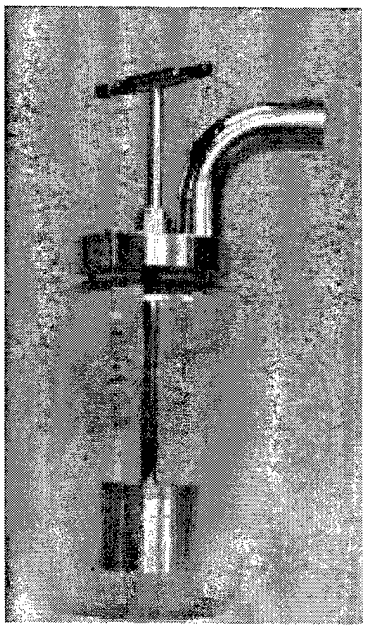

Figure 2. 24-Jet Nebulizer.[1]

2.3 Sonic Nozzles.

Dry Al_2O_3, PSL microspheres, and bioparticles are aerosolized using a two-fluid pneumatic sonic nozzle (Figure 3). Under contract with ECBC, SRI International (Menlo Park, CA) developed the initial nozzle, which is currently built at ECBC. The nozzle is connected to compressed air, and the air exits through a small annular opening. The low pressure created in the exit region causes powder to be pulled through an axial tube at a very low feed rate due to the Bernoulli effect. The desired air to powder mass ratio is 80-100:1. Because the airflow rate (1100 L/min) and the aerosol generation rate are high, particles generated by this method are highly charged and cannot be neutralized using the Kr-85 neutralizer.

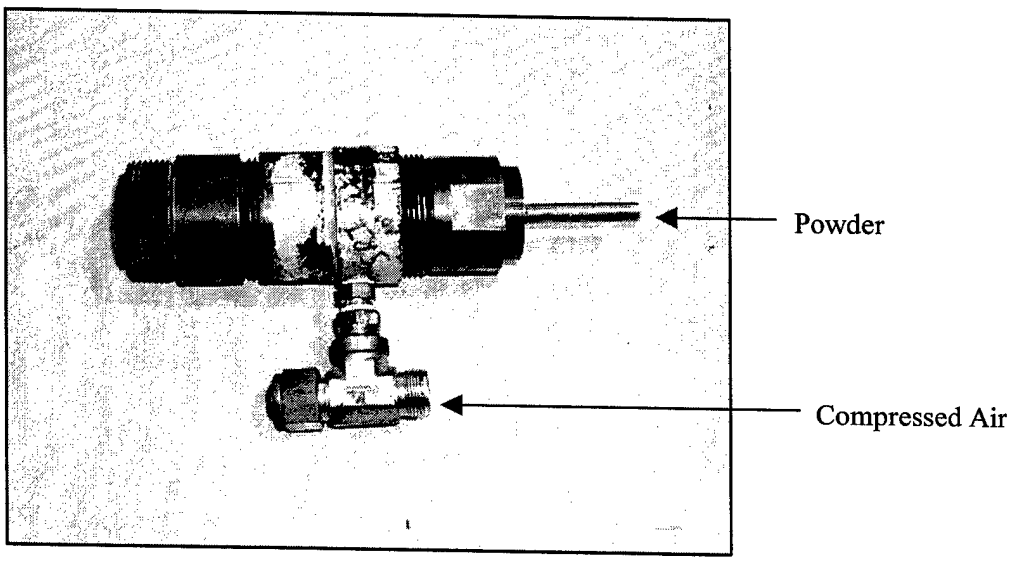

Figure 3. Sonic Nozzle.

2.4 Puffers.

Puffers are metered dose inhalers (MDI) adapted by ECBC for use with biological simulants, Figure 4. The puffer is a portable, convenient, aerosol source and ejects a small reproducible cloud of simulant aerosol particles at the push of a button. A puffer releases a 60-mg spray of its contents into the air when it is activated. The droplets expand and evaporate instantly leaving a small cloud of the loaded particles. It can be used as a quick source of particles in the laboratory and as an operation check in equipment deployed in the field. A puffer could also be used to fill a small chamber for more quantitative experiments. We have used materials such as Bg (*Bacillus subtilis var niger*), ovalbumin, and PSL microspheres in the puffers. A typical formulation is 0.1% by weight of simulant material in 1,1,1,2-tetrafluoroethane (HFA-134a) as the propellant. The shot-to-shot mass variation is small, about 10%.

Figure 4. Puffer.

2.5 Ink Jet Aerosol Generators (IJAG).

The Ink Jet Aerosol Generator (IJAG), Figure 5, was developed at ECBC for low concentration aerosol applications; however, the generator can also be used in some mid and high concentration applications. In aerosol sampler characterization tests, the IJAG is typically used to generate particles directly into the aerosol sampler.

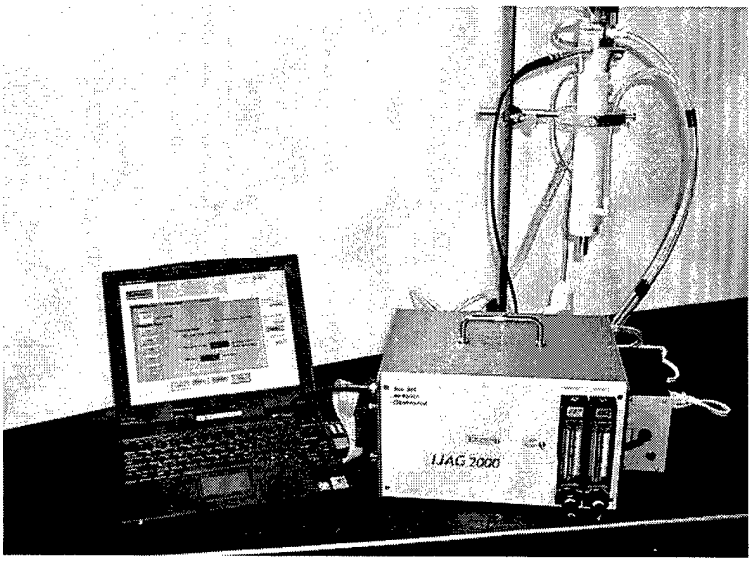

Figure 5. Ink Jet Aerosol Generator.

A 12-nozzle ink jet cartridge (HP 51612A), purchased empty, is filled with a slurry of clean water and the material of interest. Droplets are fired downward through a heated drying tube in which the water evaporates, leaving an aggregate residue particle. Since the size of the primary ink jet droplet is fixed, about 50-μm diameter, the size of the residue particle depends only on the concentration

of the slurry. We have prepared different concentrations of slurries to produce different size monodisperse particles (3-10 μm). The IJAG is capable of producing monodisperse particles at a range of 1-2,000 particles/second for our tests.

2.6 Vibrating Orifice Aerosol Generator (VOAG).

A model 3450 Vibrating Orifice Aerosol Generator (VOAG) (TSI Incorporated, St. Paul, MN), Figure 6, is used in generating monodisperse fluorescent oleic acid droplets. The generated droplets pass through a model 3054 Kr-85 neutralizer before entering the chamber to neutralize the charged particles.

Different concentrations of sodium fluorescein and oleic acid in propanol are used to generate different size particles. The solution to be aerosolized is placed in a pressure container. The pressure forces the solution at a constant flow rate from the pressure container through a membrane filter and Teflon tubing into the liquid orifice assembly. The solution is forced through a small orifice to form a liquid jet. A piezoelectric crystal produces mechanical vibration in the liquid orifice disk. The vibration causes the liquid jet to break up into uniform droplets. The alcohol evaporates, leaving fluorescent-tagged oleic acid as the final challenge particle. Changing the concentration of oleic acid in propanol, the vibration frequency of the liquid orifice disk, and the liquid feed rate can change the particle size of the aerosols.

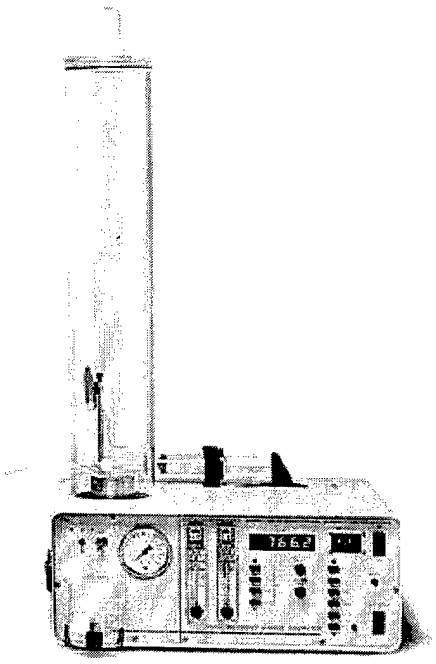

Figure 6. Vibrating Orifice Aerosol Generator.[2]

2.7 Fluorometer.

The Turner Model 450 Fluorometer (Barnstead/Thermolyne Corporation, Dubuque, IA), Figure 7, is used to measure the fluorescence of the collected samples. Appropriate excitation and emission filters are used to detect the blue (Ex:NB360; Em:SC430) and green (Ex:NB460; Em:SC500)

PSL microspheres and sodium fluorescein (Ex:NB490; Em:SC515). When measuring sodium fluorescein, the pH of the sample must be between 8-10 to achieve maximum fluorescence. The temperature also affects the amount of fluorescence; therefore, the samples that are compared should be at the same temperature.

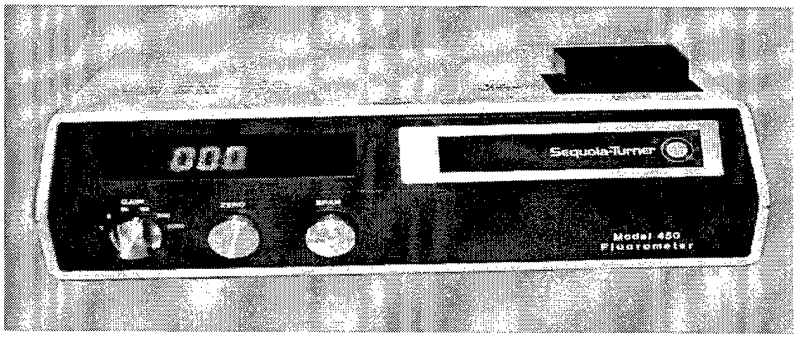

Figure 7. Fluorometer.[3]

2.8 Coulter Multisizer®.

The Coulter Multisizer® II analyzer (Beckman Coulter, Miami, FL), Figure 8, is a multichannel particle size analyzer that uses electrical impedance across a small orifice in a tube through which the particles travel in a liquid suspension as a method of measurement to provide a particle size distribution analysis. There are different size orifice tubes for measuring different ranges of particle sizes. We generally use the 50 μm aperture diameter orifice tube in the Coulter Multisizer® that measures 1-30-μm diameter particles.

The Coulter Multisizer® analysis method requires that the sample be in an electrolyte solution for analysis. This is achieved by either using the electrolyte solution as the sample collection liquid in the sampler or by diluting high concentrations of samples in electrolyte solution. Filters are processed in the electrolyte solution to remove the particles from the filter into the solution for Coulter Multisizer® analysis. The measured geometric diameter is converted to aerodynamic diameter using the density and the shape factor of the particles. The disassociation of agglomerated and clustered particles in the electrolytic is not desired and should be checked before using the Coulter Multisizer® as the analysis method.

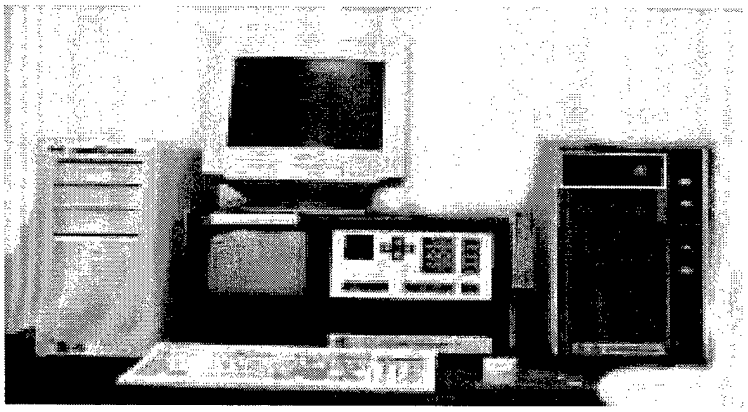

Figure 8. Coulter Multisizer® II.[4]

2.9 Aerodynamic Particle Sizer (APS).

The APS Model 3320 (TSI Incorporated, St. Paul, MN) is a high performance general purpose particle spectrometer that measures aerodynamic diameter and light-scattering intensity. The APS provides accurate particle count and size distributions for particles with aerodynamic diameters from 0.5 to 20 μm. It detects light-scattering intensity for particles from 0.3 to 20 μm. The APS is specifically engineered to perform aerodynamic size measurements in real time using low particle accelerations.

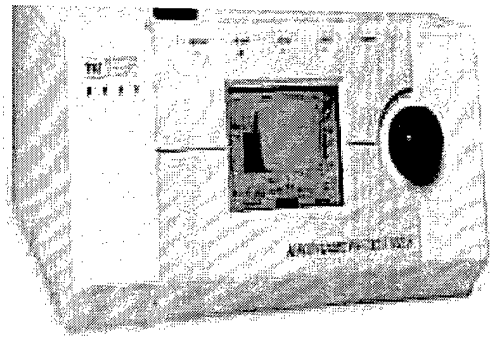

Figure 9. Aerodynamic Particle Sizer.[5]

The aerodynamic size is determined by the acceleration rate. The APS measures the acceleration of aerosol particles in response to the accelerated flow; the smaller particles accelerate faster, and the larger particles accelerate slower. As the particles exit the nozzle, the time of flight between two laser beams is recorded and converted to an aerodynamic diameter using an internal calibration table. To gain additional information, the light-scattering information is also obtained and plotted against the aerodynamic size.

3. SAMPLER EFFICIENCY TEST METHODS

3.1 Polystyrene Latex Microsphere (PSL) Tests.

Sampling efficiency tests are conducted with monodisperse fluorescent and non-fluorescent PSL microspheres (Duke Scientific Corporation, Palo Alto, CA). The PSL aerosols are generated using either a 24- or 36-jet Collison nebulizer, a sonic nozzle, an IJAG, or puffers. The PSL microspheres have to be in powder form for aerosolization using the sonic nozzle. Generally, we use the 36-jet Collison nebulizer to generate aerosols because the low airflow rate allows the aerosol to be neutralized using a Kr-85 neutralizer before the aerosol enters the chamber. During experiments, aerosols are generated for 10-20 min, and the chamber air is mixed for 1 min to achieve uniform aerosol concentration before sampling using reference filters and the samplers being tested.

To collect fluorescent PSL microspheres if the analysis is by either fluorometry or Coulter Multisizer®, polycarbonate membrane filters (Osmonics Incorporated, Minnetonka, MN) are used as reference filters. After sampling, the sample is collected from the system and reference filters being tested. Sample liquids are directly analyzed by either fluorometry or Coulter Multisizer®; however, the membrane filters and any dry samples from systems being tested are processed to remove microspheres from the filters and surfaces into the liquid for either fluorometer or coulter analysis. The removal procedure consists of placing the membrane filters into 20 mL of filtered deionized water,

shaking the solution by hand for 30 s, and vortexing the test tubes in a holder for 30 min. The sample is removed from the vortexer every 10 min and shaken by hand (Kesavanathan and Doherty, 1999).[6]

3.2 Polydisperse Solid Aerosol Tests.

Polydisperse Al_2O_3 particle (Saint-Gobain Industrial Ceramics, Worcester, MA) aerosol, Figure 10, is generated using the sonic nozzle in the 70-m^3 chamber. One gram of powder takes approximately 10-15 s to become aerosolized. The generated aerosol is mixed in the chamber for 1 min before sampling. Polycarbonate membrane filters (Osmonics Incorporated, Minnetonka, MN), as described above, are used as reference filters. Particles collected on reference filters are removed from the filters as described in PSL tests. Collected samples are analyzed using the Coulter Multisizer®. The measured geometric diameter of Al_2O_3 by the Coulter Multisizer® is converted to aerodynamic diameter using the density of Al_2O_3 (4 g/cm^3) and the shape factor (1.22). Currently, the aerosols are not neutralized; however, we are in the process of designing and building a neutralizer for the sonic nozzle.

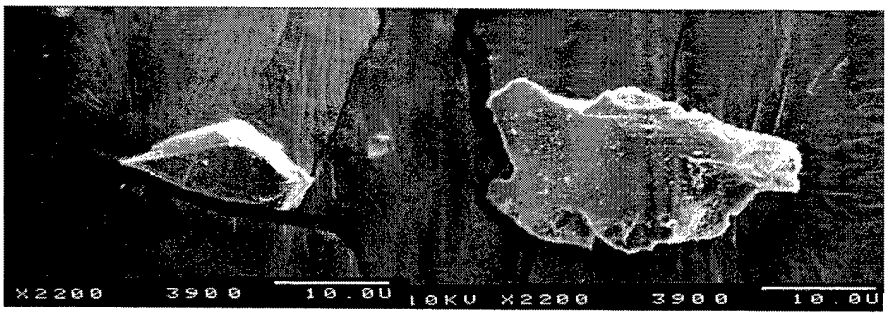

Figure 10. Al_2O_3 Particles.

3.3 Sodium Fluorescein-Tagged Oleic Acid (Fluorescent Oleic Acid) Tests.

Monodisperse fluorescent oleic acid particles, Figure 11, are generated using a VOAG, which can be used to generate monodisperse 3- to >20-μm diameter particles. Sampling the aerosol onto a microscope slide inserted into an impactor, and measuring droplet size using a microscope determines the sizes of the fluorescent oleic acid particles. The measured fluorescent oleic acid particle diameter is converted to an aerodynamic particle size using a spread factor and the density of fluorescent oleic acid. At the end of aerosol generation, the aerosol in the chamber is mixed for 1 min to achieve a uniform aerosol concentration. The samplers and the corresponding reference filters sample the aerosol simultaneously for the same amount of time.

Glass fiber filters (Pall Corporation, Ann Arbor, MI) are used as the reference filters to collect the fluorescent oleic acid particles. After sampling, the filters are removed from filter holders, placed into a fluorescein recovery solution, and shaken on a table rotator (Lab-Line Instruments, Incorporated, Melrose Park, IL) for 1 hr. The recovery solution used in the tests is water with a pH between 8 and 10, obtained by adding a small amount of NH_4OH (e.g., 1000 mL of water with 0.563 mL of 14.8 N NH_4OH). Kesavan et al. (2001)[7] describe in detail factors that affect fluorescein analysis and the removal of fluorescein from filters. The samples from the aerosol samplers were pH corrected by adding NH_4OH before the amount of fluorescence was measured by the fluorometer (Barnstead/ Thermolyne, Dubuque, IA). All the samples are analyzed either the day of or the day after the experiment. In appropriate situations, the recovery solution is used as the sample collection liquid. Over a 12-day period, separate tests did not show any photo degradation of fluorescence with time under normal laboratory lighting.

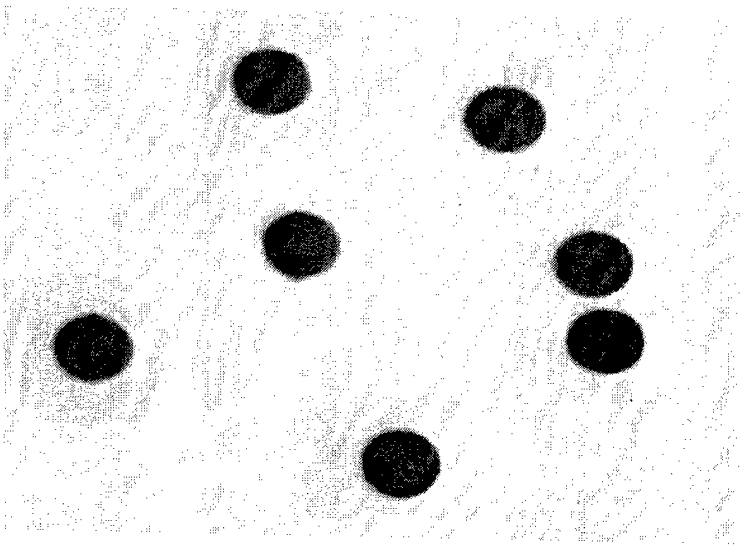

Figure 11. Microscopic Picture of 10-μm Aerodynamic Diameter Fluorescent Oleic Acid Droplets.

3.4 Bioaerosol Tests.

Clustered monodispersed bioparticles are generated using an IJAG. Polydispersed biological particles are generated using puffers, nebulizers, and the sonic nozzle. Analyses are by culturing, PCR, APS, ELISA, and/or Coulter Multisizer®. Similar to other methods, the aerosol is generated for a certain time, and chamber air is mixed for 1 min to obtain a uniform aerosol concentration in the chamber. The inkjet aerosol generator output can either go into the inlet of the sampler or into a small chamber for sampling efficiency tests. The reference filters and samplers being characterized sample the aerosol simultaneously for the same amount of time. At the end of sampling, the reference filters and samples are collected for analysis. Membrane filters are used to collect the bioparticles if the analysis method is by Coulter Multisizer®. Samples are sent out for analyses by culturing, ELISA, and/or PCR.

4. DISCUSSION

The use of polydisperse solid aerosols with a Coulter Multisizer® analysis gives all particle size information at once. Therefore, it is used as a quick test to characterize samplers over a range of particle sizes and identify the size range of interest for more detailed, accurate, and time-consuming study using monodisperse particles. Solid particles may bounce when they hit a surface and reach the collection site; on the other hand, liquid particles are removed from the air when they hit surfaces and may not reach the collection site. After an initial quick test, if the sampler has good sampling efficiency, additional detailed sampling efficiency tests using monodisperse fluorescent oleic acid particles are conducted with fluorometer analysis. Sampling efficiency tests using monodisperse particles are conducted one particle size at a time – a time-consuming procedure.

To improve sample recovery, membrane filters must be used as a filter medium to collect the solid particles for Coulter Multisizer® and fluorometer analyses. However, the airflow rate through the membrane filters cannot be very high compared to the glass fiber filters.

The use of PSL microspheres is a convenient and accurate method for monodisperse small particle (0.5-3 μm) tests. They are easy to aerosolize but are expensive. The PSL beads have a limited amount of fluorescence. Therefore, very small amounts cannot be detected by the fluorometer. Also, for Coulter Multisizer® analysis, the particle count has to be significantly above background noise.

The use of fluorescent oleic acid with fluorometric analysis is an accurate method for 3-20 μm diameter particle tests. It is difficult to generate particles smaller than 3 μm using the VOAG. Correct excitation and emission filters should be installed in the fluorometer to detect sodium fluorescein. Samples have to be pH corrected for the maximum fluorescence of sodium fluorescein. All the samples have to be at the same temperature because temperature affects the amount of fluorescence (Kesavan et al., 2001).[7]

Care must be taken in using bioaerosols in sampler characterization tests. Many aerosol generation and sampling methods kill vegetative bacteria and some spores. Another disadvantage is that some organisms start growing once they are mixed with the right nutrients; therefore, care must be taken to avoid contact with nutrient material during sampling. Samples also have to be refrigerated to prevent organism growth if the analysis is planned for a later time.

Tests with no aerosols are conducted to determine background measurements of the samplers as well as reference filters. In addition, prewashes are conducted before each test to confirm that the samplers are free of test materials. After the first sampling test, up to four washes are conducted to remove test material from the samplers, and to determine the number of washes required to remove all test material from the sampler after each test. Based on these results, the samplers are washed in between tests to remove all test materials from the samplers.

5. CONCLUSIONS

Dry aerosols, wet aerosols, and bioaerosols have different transmission and collection efficiencies. Dry particles bounce from surfaces when they impact onto surfaces, and wet particles are completely captured. Biological particles fall in between solid and liquid particles on their characteristics of bounce. We routinely characterize samplers with all three aerosols. Samplers are characterized with 1-10-μm particles to determine the sampling efficiency and are characterized with >10-μm particles to determine the sampler's rejection efficiency of larger particles. The lower limit of detection is tested using the Ink Jet Aerosol Generator.

Samplers are routinely characterized in chambers at the U.S. Army Edgewood Chemical Biological Center (ECBC) at calm air conditions using the following methods: (1) monodisperse fluorescent/nonfluorescent Polystyrene Latex microspheres with either fluorometric or Coulter Multisizer® analysis, (2) polydisperse solid aluminum oxide particles with either Aerodynamic Particle Sizer (APS) or Coulter Multisizer® analysis, (3) fluorescent oleic acid particles with fluorometric analysis, and (4) bioparticles with Coulter Multisizer®, culturing, enzyme linked immunosorbent assays, polymerase chain reaction, and APS analyses.

Customers and researchers request information on test aerosols, generation methods, and analysis techniques used at ECBC to characterize samplers. This information is used to replicate tests at other facilities and to prepare for sampler characterization tests at ECBC. Therefore, this methodology report is written to give customers and researchers information on sampler characterization methods used at ECBC. Such work and writing this report are funded by the ECBC tech base project.

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